Figure 1: Real-time PCR data using the Taqman probe

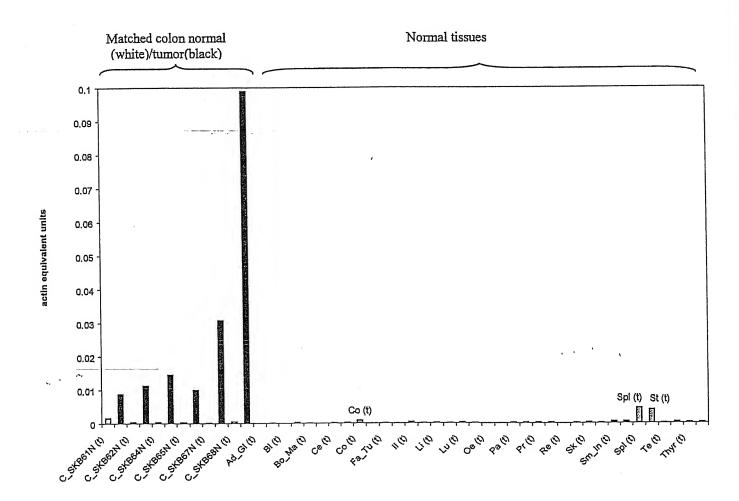


Figure 2: Real-time PCR expression using Sybr protocol

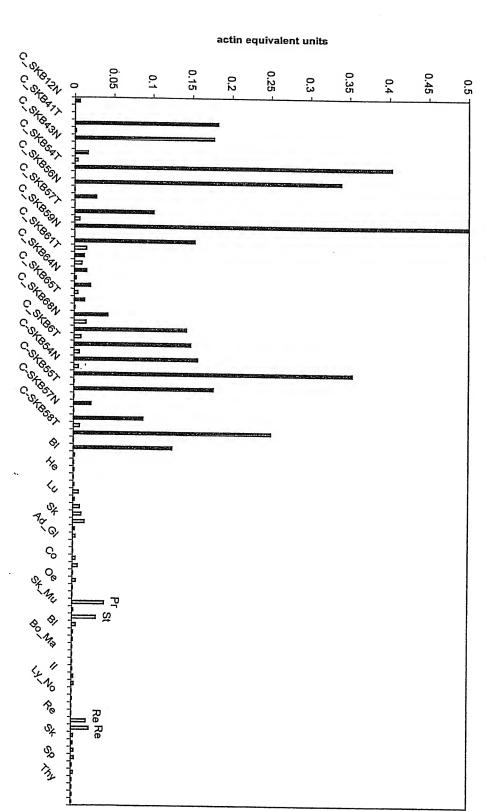
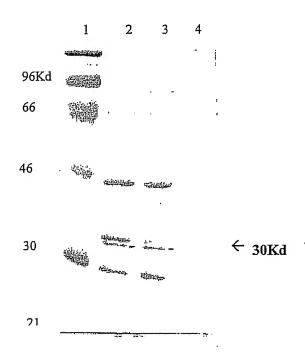


Figure 3: Coomassie blue stained SDS PAGE of the cell extract from the strain expressing CASB7439



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Figure 4: Western blot loaded with cell extract from the strain expressing CASB7439

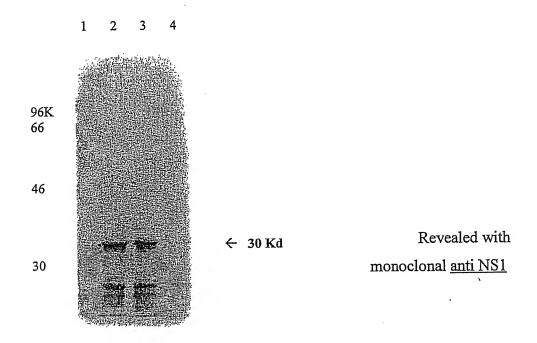


Figure 5: Coomassie-blue stained SDS-PAGE of CASB7439 after purification

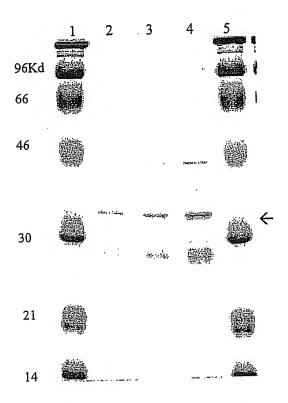
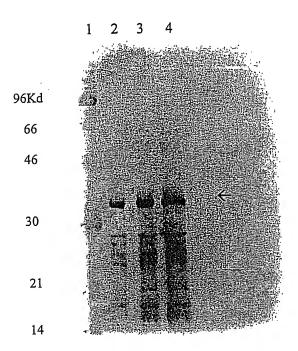


Figure 6: Western blot CASB7439 after purification (monoclonal anti-polyhistidine)



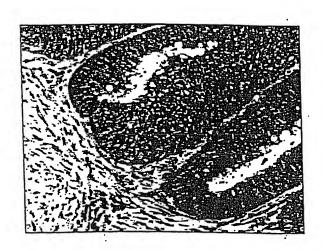


Figure 7
CASB7439 IHC on a Colon Tumour Biopsy

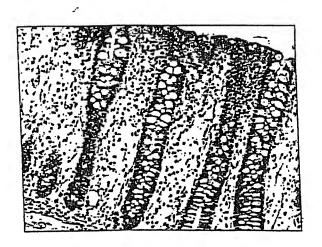


Figure 8 CASB7439 IHC on a Colon Normal Mucosa Biopsy

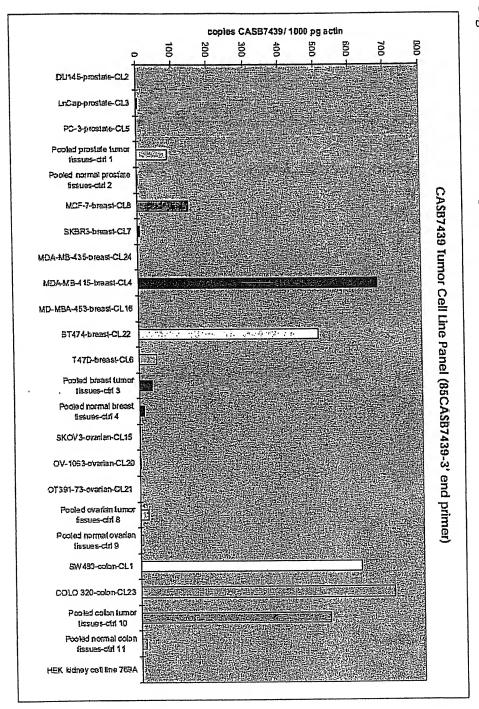
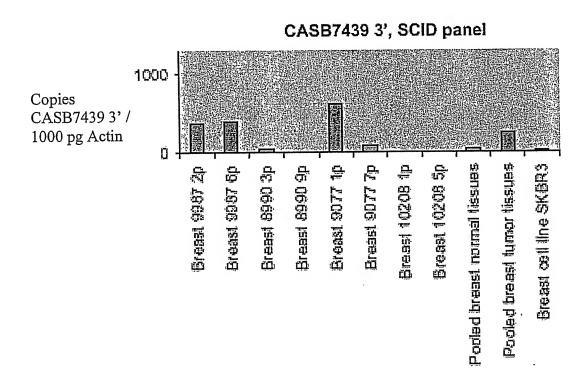


Figure.9: Real-time RT-PCR quantification of CASB7439 transcript on a panel of cell lines, tumor and normal tissues.

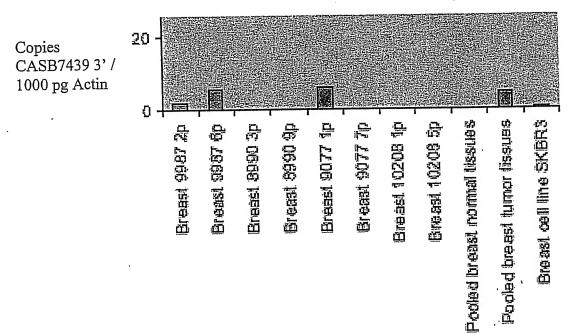
Figure 10: Real-time RT-PCR quantification (3' UTR pimers) of CASB7439 transcript on a panel of tumours grown in SCID mice.



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Figure 11: Real-time RT-PCR quantification (ORF primers) of CASB7439 transcript on a panel of tumours grown in SCID mice.

#### CASB7439 ORF, SCID panel



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Figure 12: Real-time RT-PCR quantification (3' UTR pimers) of CASB7439 transcript on an extended panel of normal and tumours tissues.

### CASB7439 3', Extended Breast Panel

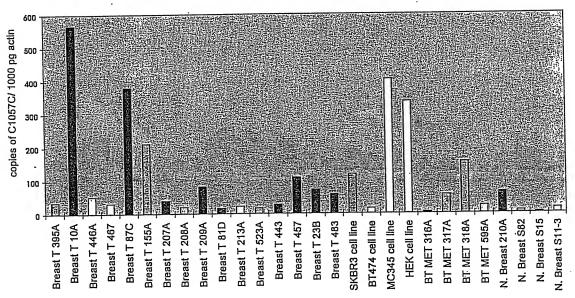
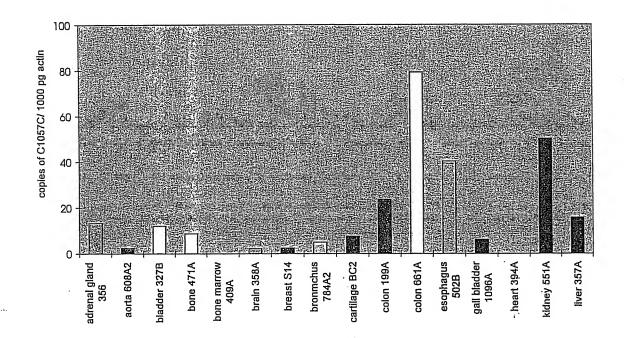
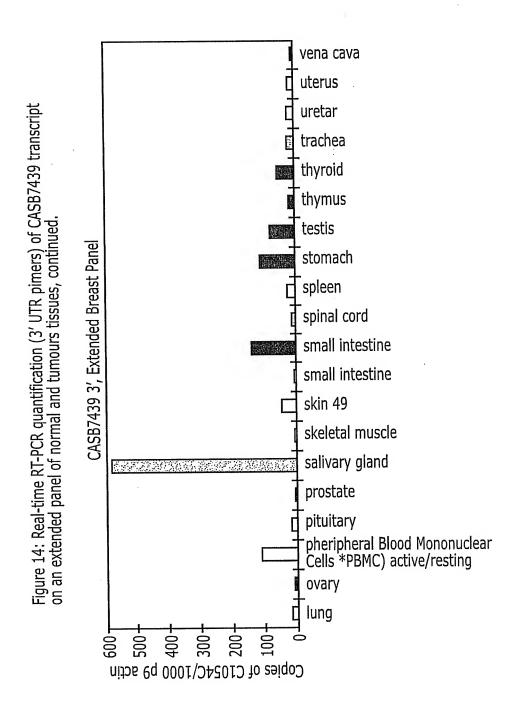


Figure 13: Real-time RT-PCR quantification (3' UTR pimers) of CASB7439 transcript on an extended panel of normal and tumours tissues.





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Figure 15: Design of NS1-CASB7439 protein.

N term	NS1		CASB7439		HIS	C term
1		 1		194		
8	0					

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Figure 16: Schematic represention of pMG1 cloning strategy (constructs 1 and 2).

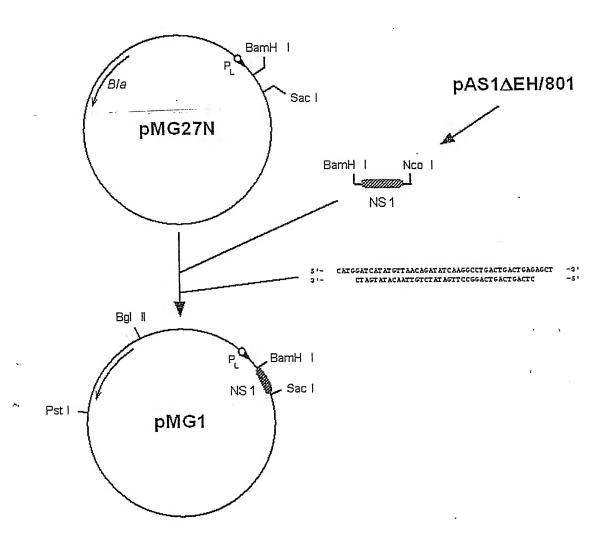
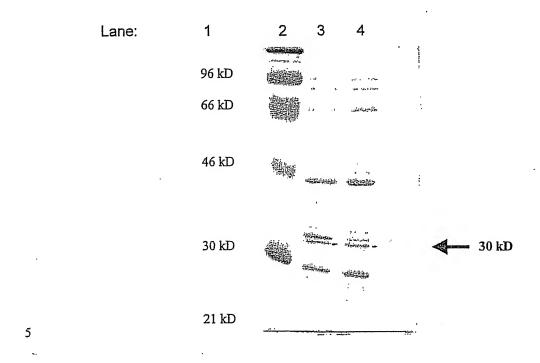


Figure 17: Coomassie blue stained SDS PAGE of the cell extract from the strain expressing CASB7439 construct 1.



Legend:

Lane 1: Molecular weights

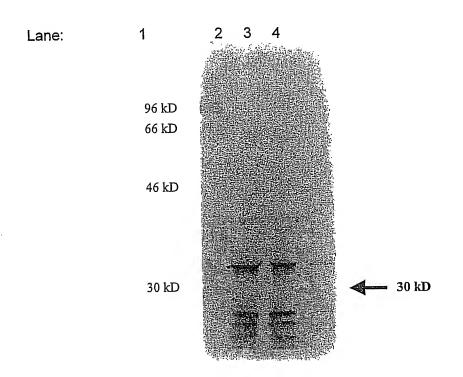
Lane 2: Whole cellular extract

10 Lane 3: Supernatant of cellular extract

Lane 4: Pellet of cellular extract

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Figure 18: Western blot loaded with cell extract from the strain expressing CASB7439 construct 1.



#### Legend:

Lane 1: Molecular weights

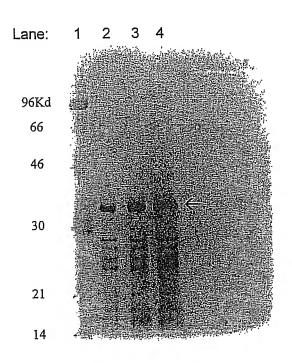
Lane 2: Whole cellular extract

Lane 3: Supernatant of cellular extract

Lane 4: Pellet of cellular extract

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Figure 19: Western blot CASB7439 construct 1 after purification (monoclonal anti-NS1 N terminal fragment).



#### Legend:

Lane 1: Molecular weights

Lane 2: 2 µl of purified protein

Lane 3: 4 µl of purified protein

Lane 4: 6 µl of purified protein

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Figure 20: Comparison of Constructs 3 to 5 expression yields. Western blot on CASB7439 constructs 3 to 5 expressed in E. coli, revealed with antipeptide SB600 antibody.

,	Cc	nstru	uct 3			Construct 4					Construct 5			
4	2	3	ч	5	6	4	8	3	lo	AA	42	<b>A3</b>	Α¥	
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81													•	
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<b>US</b>	Ì.,					- 17 - 171·	5 550/21 F				÷			
<b>14:5</b>						南海南南南北	rita							

#### Legend:

Lane 1: MW; Kd

Lane 2:Construct 2, undiluted.

Lane 3: Construct 2, 1/10 dilution.

Lane 4: Construct 2, 1/50 dilution.

Lane 5: Construct 2, 1/100 dilution.

Lane 6: Construct 3, undiluted.

Lane 7: Construct 3, undiluted.

Lane 8: Construct 3, 1/10 dilution.

Lane 9: Construct 3, 1/50 dilution.

Lane 10: Construct 3, 1/100 dilution.

Lane 11: Construct 4, undiluted.

Lane 12: Construct 4, 1/10 dilution.

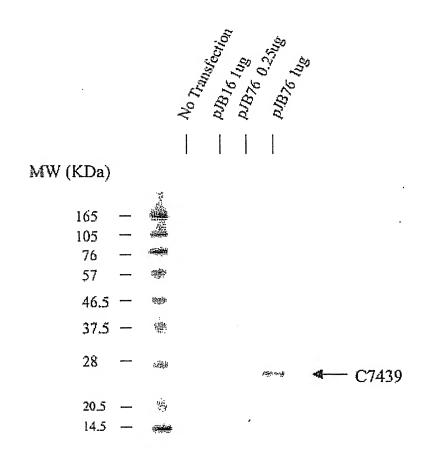
Lane 13: Construct 4, 1/50 dilution.

Lane 14: Construct 4, 1/100 dilution.

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Figure 21: Expression of C7439 in HEK 293T Cells by DNA Vaccine Vector pJB76 (pJB16/C7439)

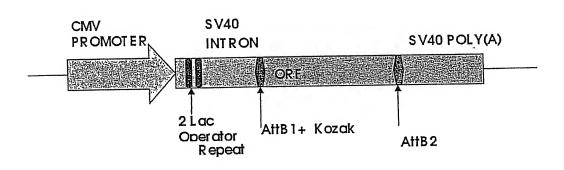
### Expression of C7439 in HEK 293T Cells by DNA Vaccine Vector pJB76 (pJB16/C7439)



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Figure 22: Schematic representation of CASB7439 expressed in a adenoviral live vector

### Adenovirus Lac/GW-1 Expression Cassette



AttB1 ACAAGTTT atacaaaAAAGC AttB2 ACCCAGCTTT cttatacAAA

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Figure 23: Expression of C7439 in Human Fibroblasts by Recombinant Adenovirus AdC7439

### Expression of C7439 in Human Fibroblasts by the Recombinant Adenovirus AdC7439

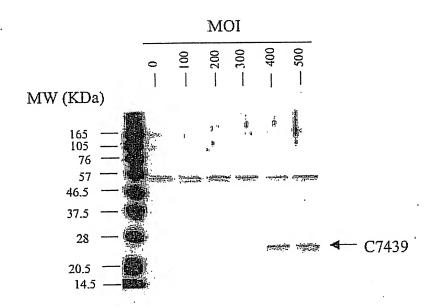


Figure 24: Real-time RT-PCR quantification of CASB7439 transcript on a population of preneoplasic lesions of lung (PLL) patients.

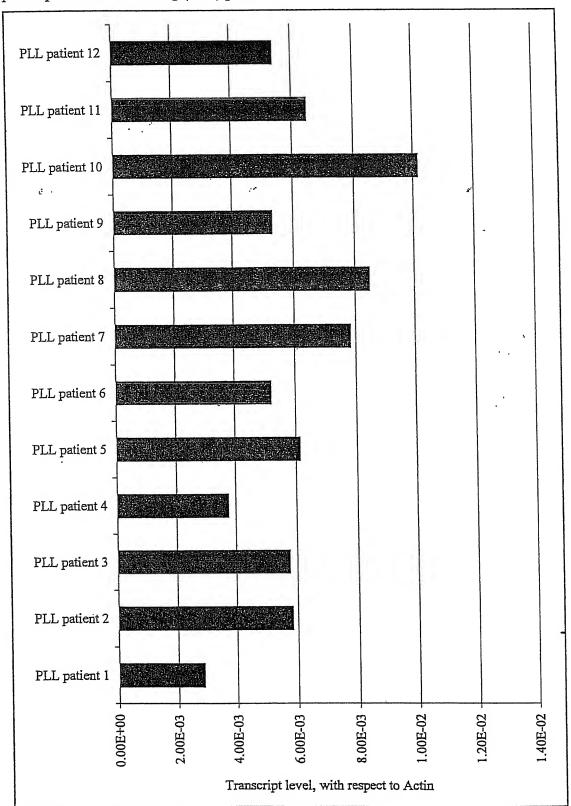


Figure 25: Real-time RT-PCR quantification of CASB7439 transcript on a population oc early stage lung cancer (ESLC) patients.

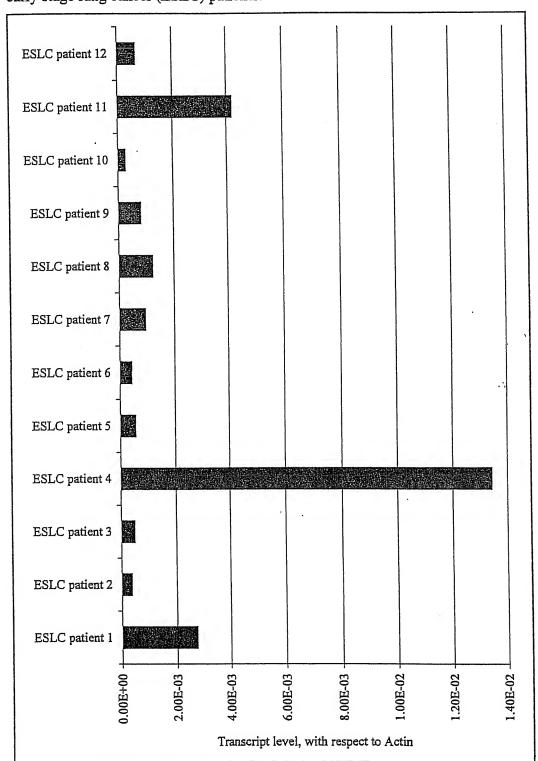


Figure 26: Real-time RT-PCR quantification of CASB7439 transcript on a population of late stage lung cancer (LSLC) patients.

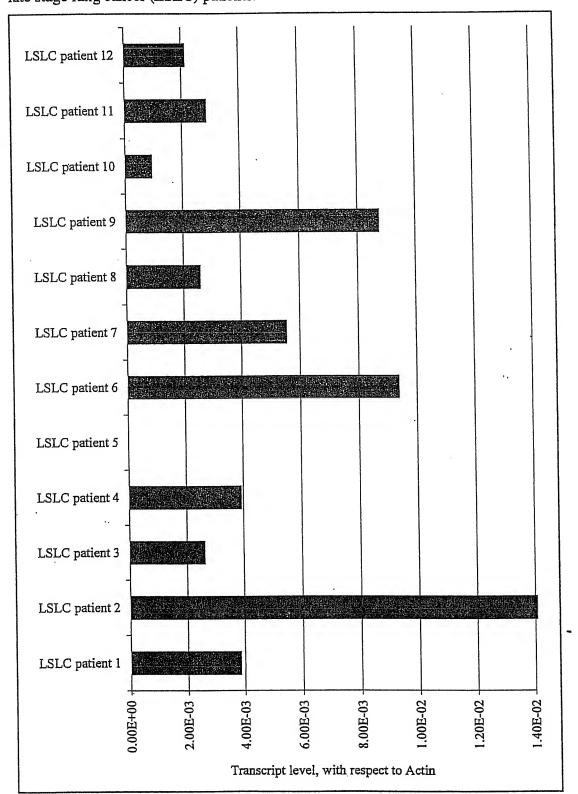


Figure 27: Real-time RT-PCR quantification of CASB7439 transcript on a panel of normal tissues.

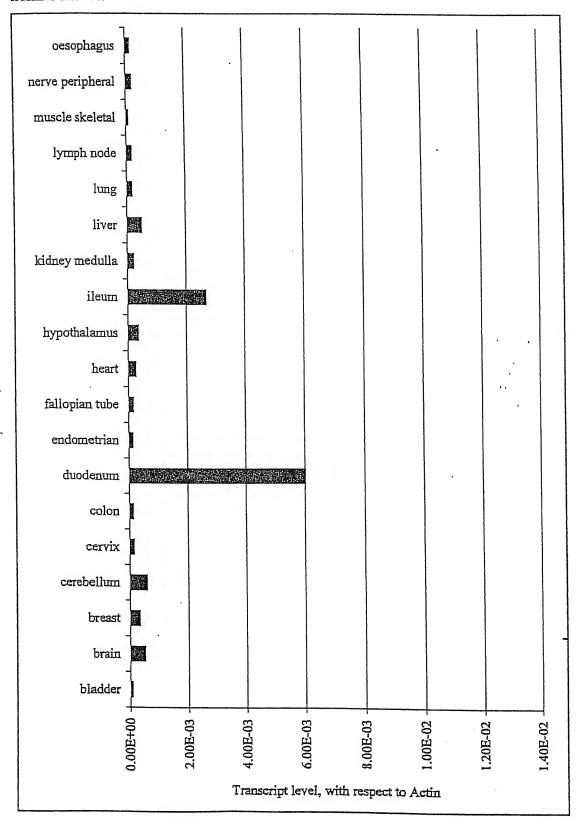


Figure 28: Real-time RT-PCR quantification of CASB7439 transcript on a panel of normal tissues, continued.

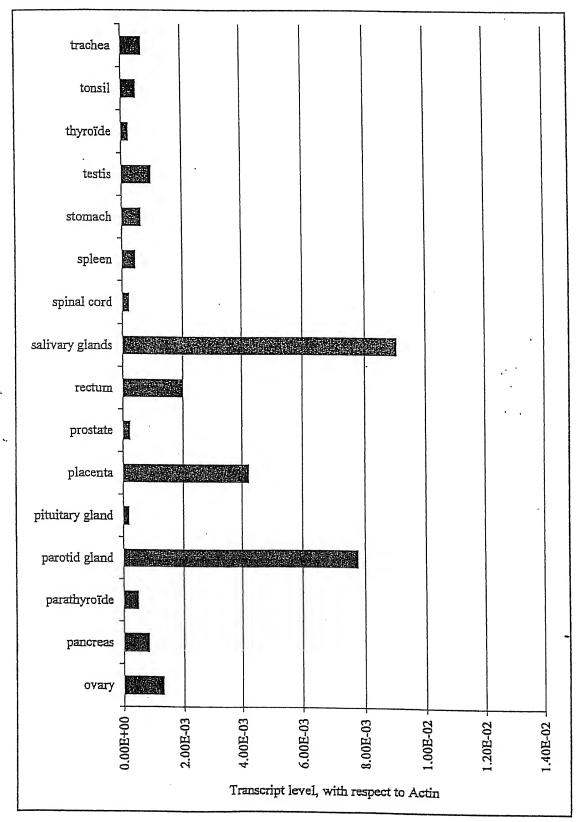


Figure 29: Design of NS1-CASB7439 protein.

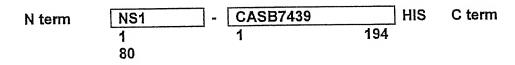
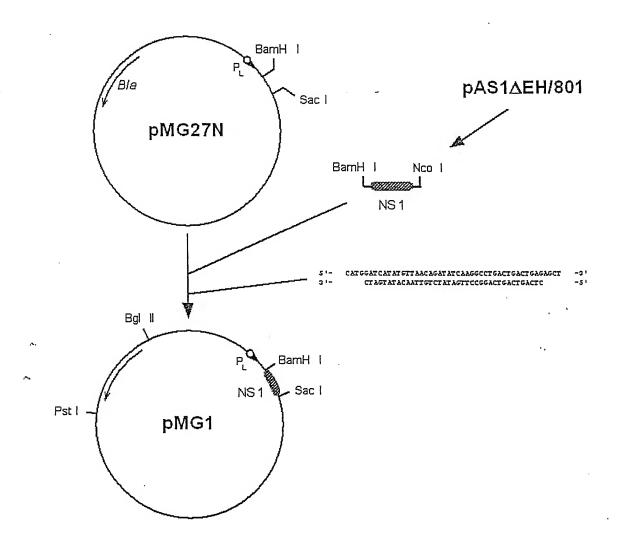
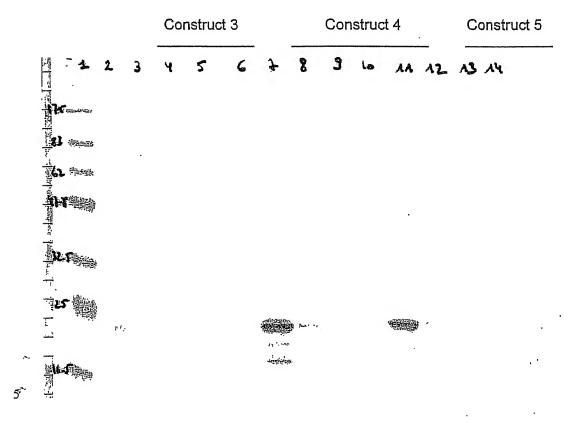


Figure 30: Schematic represention of pMG1 cloning strategy (constructs 1 and 2).



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Figure 31: Comparison of Constructs 3 to 5 expression yields. Western blot on CASB7439 constructs 3 to 5 expressed in *E. coli*, revealed with antipeptide SB600 antibody.

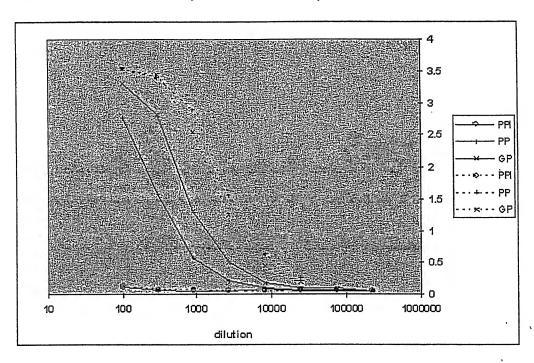


#### Legend:

- Lane 1: MW; Kd
- Lane 2: Construct 2, undiluted.
- Lane 3: Construct 2, 1/10 dilution.
- Lane 4: Construct 2, 1/50 dilution.
- Lane 5: Construct 2, 1/100 dilution.
- Lane 6: Construct 3, undiluted.
- Lane 7: Construct 3, undiluted.
- Lane 8: Construct 3, 1/10 dilution.
- Lane 9: Construct 3, 1/50 dilution.
- Lane 10: Construct 3, 1/100 dilution.
- Lane 11: Construct 4, undiluted.
- Lane 12: Construct 4, 1/10 dilution.
- Lane 13: Construct 4, 1/50 dilution.
- Lane 14: Construct 4, 1/100 dilution.

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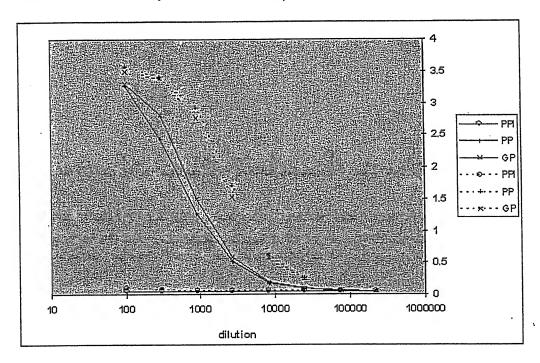
Figure 32: CASB7439 peptide 1 immunization of rabbit SB598: graphical view of ELISA read-outs (Antibody titers relative to CASB7439 peptide 1, straight line; antibody titers relative to KLH carrier, discontinuous line)



5<sub>x.</sub>

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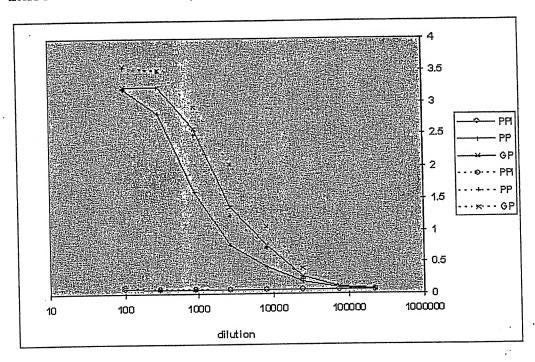
Figure 33: CASB7439 peptide 1 immunization of rabbit SB599: graphical view of ELISA read-outs (Antibody titers relative to CASB7439 peptide 1, straight line; antibody titers relative to KLH carrier, discontinuous line).



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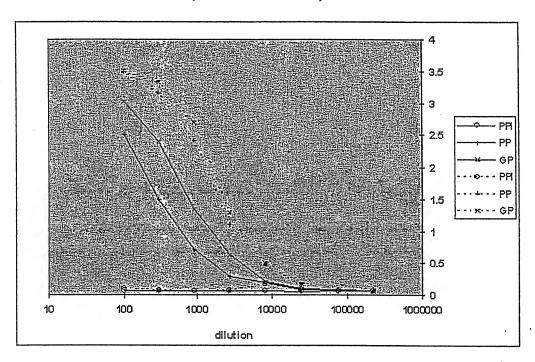
Figure 34: CASB7439 peptide 2 immunization of rabbit SB600: graphical view of ELISA read-outs (Antibody titers relative to CASB7439 peptide 2, straight line; antibody titers relative to KLH carrier, discontinuous line).



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Figure 35: CASB7439 peptide 2 immunization of rabbit SB601: graphical view of ELISA read-outs (Antibody titers relative to CASB7439 peptide 2, straight line; antibody titers relative to KLH carrier, discontinuous line).



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